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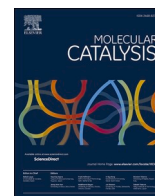
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Chemoenzymatic intermolecular haloether synthesis

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ABSTRACT

A chemoenzymatic method for the synthesis of haloethers is presented. A combination of enzymatic hypohalite synthesis with spontaneous oxidation of alkenes and nucleophilic attack by various alcohols enabled the synthesis of a wide range of haloethers. The reaction system has been characterised and current imitations have been worked out. In the present, aqueous reaction system, hydroxyhalide formation represents the main undesired side reaction. Nevertheless, semi-preparative scale synthesis of a range of haloethers is demonstrated.

Introduction

Ether synthesis is one of the oldest and most fundamental transformations in organic chemistry. Discovered in the mid-19th century, the Williamson ether synthesis and its modifications such as the Ullmann biaryl ether synthesis now are well established in chemical education and on industrial scale.[1] Next to organic halides also C=C-double bonds principally can function as electrophiles to form (substituted) ethers with alcohols. However, unless the C=C-double bond is activated e.g. through conjugation with carbonyl groups (Michael addition), it requires activation, mostly through Lewis- or Brønsted acids, to react with alcohol nucleophiles. The rather harsh, acidic conditions pose a considerable challenge for functional group tolerance of these methods. Oxidative activation of C=C-double bonds represents an interesting alternative as these methods generally result in a bifunctionalisation of the original C=C-double bond and thereby open up possibilities for further follow-up reactions (Scheme 1). Epoxidation of alkenes followed by nucleophilic ring opening as well as halofunctionalisation of alkenes using (in situ generated) electrophilic halide species represent the methods of choice here.

Various reagents for the controlled release of electrophilic halide species are available such as N,N'-dibromobenzene sulphonamide, selectfluor, p-nitrobenzene-sulphonyl peroxide/Br⁻, tribromoisocyanuric acid, Ag⁺/Br₂, NBS/acid/MeOH, or 1,3-dibromo-5,5-dimethylhydantoin (DDH)/MeOH.[2] These procedures, however, suffer from large waste generation not only negatively impacting their

waste footprint but also complicating downstream processing. *In situ* generation of hypohalites from corresponding halides and H_2O_2 represents one of the most atom-economic approaches as only water is generated as waste product. Recently, we have established a chemoenzymatic approach for the hydroxyhalogenation of alkenes using the vanadium-dependent haloperoxidase from *Curvularia inaequalis* (*Ci*VCPO).[3–6] Therein, *Ci*VCPO catalyses the H_2O_2-dependent oxidation of halides such as Cl⁻, Br⁻ and I⁻ with the resulting hypohalites reacting spontaneously with a broad range of C=C-double bonds.[7] This reaction can be extended to intramolecular lactonisation/etherification replacing water as nucleophile.[8–11] An intermolecular variant appeared unlikely due to the overwhelming presence of water as nucleophile.

Nevertheless, we set out to explore the possibility of intermolecular etherification in a chemoenzymatic approach outlined in Scheme 2.

Experimental

Preparation of CiVCPO

Heterologous expression and purification of the chloroperoxidase from *Curvularia inaequalis* (*Ci*VCPO) were performed following established procedures:[6] A 2 L culture of *Escherichia coli* transformant (*E. coli* TOP10 (Invitrogen) with the construct *pBAD-VCPO*) was grown at 37 °C in LB medium supplemented with 100 µg/mL ampicillin to an OD 600 nm between 0.6 and 0.8. Protein expression was induced after

A) Epoxidation/Nucleophilic ring opening strategy

B) Oxidative halogenation strategy
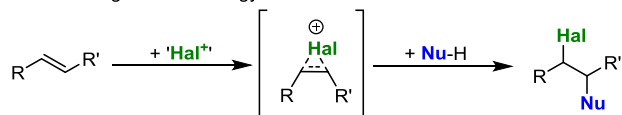
Scheme 1. Oxidative activation strategies for alkenes. Generally, the epoxidation/nucleophilic ring opening approach is performed in two steps whereas the activation/addition reaction via halonium ions occurs as one-pot-one-step cascade.

the fermentation broth was cooled to 20 °C by addition of 0.02% L arabinose. The incubation continued for 24 h at 25 °C before cell harvest.

CiVCPO catalysed (halo)ether synthesis

A typical procedure for the haloether synthesis was as following: in a 2.0 mL reaction vial, the substrates, enzymes, halide sources and H_2O_2 were added into citrate buffer. The enzyme was added in the last step.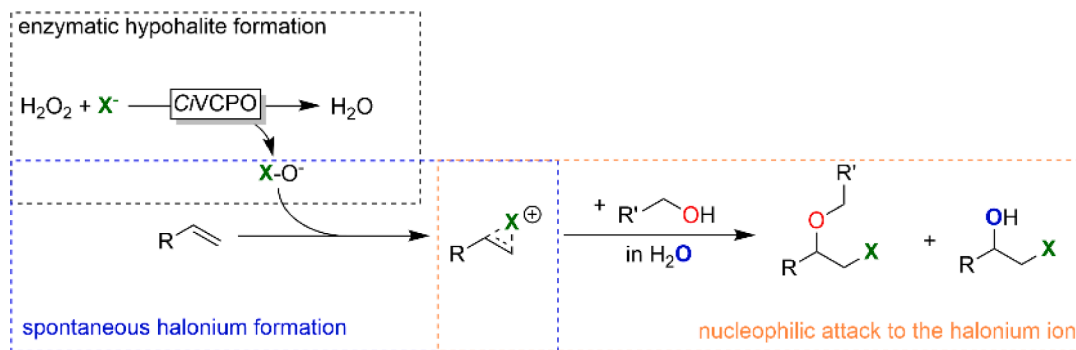 The reaction volume was adjusted to 1.0 mL. Typical ingredients of the reaction were: [styrene] = 30 mM, $[H_2O_2]$ = 100 mM, [KBr] = 100 mM, [CiVCPO] = 500 nM, pH 5.0 (citrate buffer, 100 mM), 30% of isopropanol. The reaction mixture was sealed and incubated in a thermal shaker at 800 rpm at 30 °C. At interval, aliquots were withdrawn, extracted with ethyl acetate (extraction ratio: 1:2) containing dodecane as internal standard (5 mM) and dried over Na_2SO_4. The samples were then analysed by gas chromatography (GC).

Semi-preparative synthesis of halogenated ether compounds

To 31.5 mL of citrate buffer (pH 5.0, 100 mM) in a 100 mL glass bottle, 13.5 mL of alcohol, 0.93 mL of H_2O_2 solution (5.38 M), 0.59 g of KBr and 0.15 g of styrene were added. In a final step, 150 μL CiVCPO (stock solution concentration: 13.5 mg mL^{-1}) was added. The reaction was stirred in a water bath at 30 °C for 3 h. At the end of the reaction, the organic compounds were extracted by using ethyl acetate (2 × 100 mL). The organic phases were combined and dried over anhydrous Na_2SO_4. The organic phases were then evaporated under reduced pressure. Separation was performed by column chromatography to obtain the target compound. The eluent was 1% ethyl acetate in petroleum ether solution. The isolated yield was calculated based on the ratio of the actual and theoretical amount of the ether product.

Reductive dehalogenation of the synthesised haloethers

In a 50 mL round-bottomed reaction flask, the haloether substrate (0.083 mmol) was dissolved in benzene (3 mL), followed by the addition of n-Bu$_3$SnH (72 mg, 0.25 mmol) and AIBN (41 mg, 0.25 mmol). The reaction mixture was thoroughly degassed. Finally, the reaction was performed under nitrogen atmosphere refluxing at 90 °C for 1–2 h.[12] After the completion of the reaction, the product was analysed by GC–MS. The yield was calculated on the basis of the peak area of between substrate and product in GC–MS (yield = Area$_{(product)}$ / [Area$_{(substrate)}$ + Area$_{(product)}$)]×100%).

Results and discussion

To test our hypothesis, we first investigated the oxidative haloetherification of styrene with isopropanol using CiVCPO[3-5, 13] as catalysts for the in situ formation of hypobromite. While all relevant negative controls (i.e. performing the reaction in the absence of either the catalytically active enzyme, potassium bromide or hydrogen peroxide) failed to produce any product, already the first experiment yielded significant amounts of the desired (2-bromo-1-isopropoxyethyl) benzene (0.8 mM) with the expected yet undesired water addition product (2-bromo-1-phenylethan-1-ol, 15 mM) as main product after 24 h (Figure S1).

We therefore further investigated the influence of various reaction parameters (particularly the reaction pH, concentrations of oxidant, biocatalyst and of isopropanol) on the conversion and the selectivity of the chemoenzymatic haloetherification reaction. Variation of the pH of the reaction mixture (Figure S2) revealed an optimal pH for the reaction around 5–6, which is in line with previous observations on the pH-dependency of CiVCPO-catalysed hypohalite formation [3]. The product formation rate directly correlated with the biocatalyst concentration (Figure S3) and an optimal H_2O_2 concentration between 50 and 100 mM was found (Figure S4). The somewhat unexpected activity decrease at elevated H_2O_2 concentrations most likely is due to the spontaneous reaction of H_2O_2 with hypobromite resulting in disproportionation of the oxidant[14] and formation of singlet oxygen rather than oxidative inactivation of the biocatalyst.[15]

The chemoselectivity (haloether- vs. hydroxyhalide-formation) of the overall reaction correlated with the concentration of the alcohol nucleophile applied (Fig. 1). While in the presence of low alcohol concentrations (isopropanol or methanol) predominantly the halohydrin product was formed, the ratio between halohydrin and haloethers shifted to more favourable values at elevated alcohol concentrations. In case of methanol, the desired haloether was practically the sole product above methanol concentrations of 60% (v/v). The absolute yield in haloether, however, culminated at lower alcohol concentrations (between 20 and 40% v/v). This can be attributed to the decreasing

Scheme 2. Envisioned chemoenzymatic, oxidative haloetherification of alkenes. An in situ generated halonium ion reacts with nucleophiles to form either the hydratation product or the desired ether product. The halonium ion is formed by the reaction of an alkene with hypohalites; the latter being formed enzymatically from H_2O_2 and halides using the V-dependent haloperoxidase from *Curvularia inaequalis* (*Ci*VCPO).
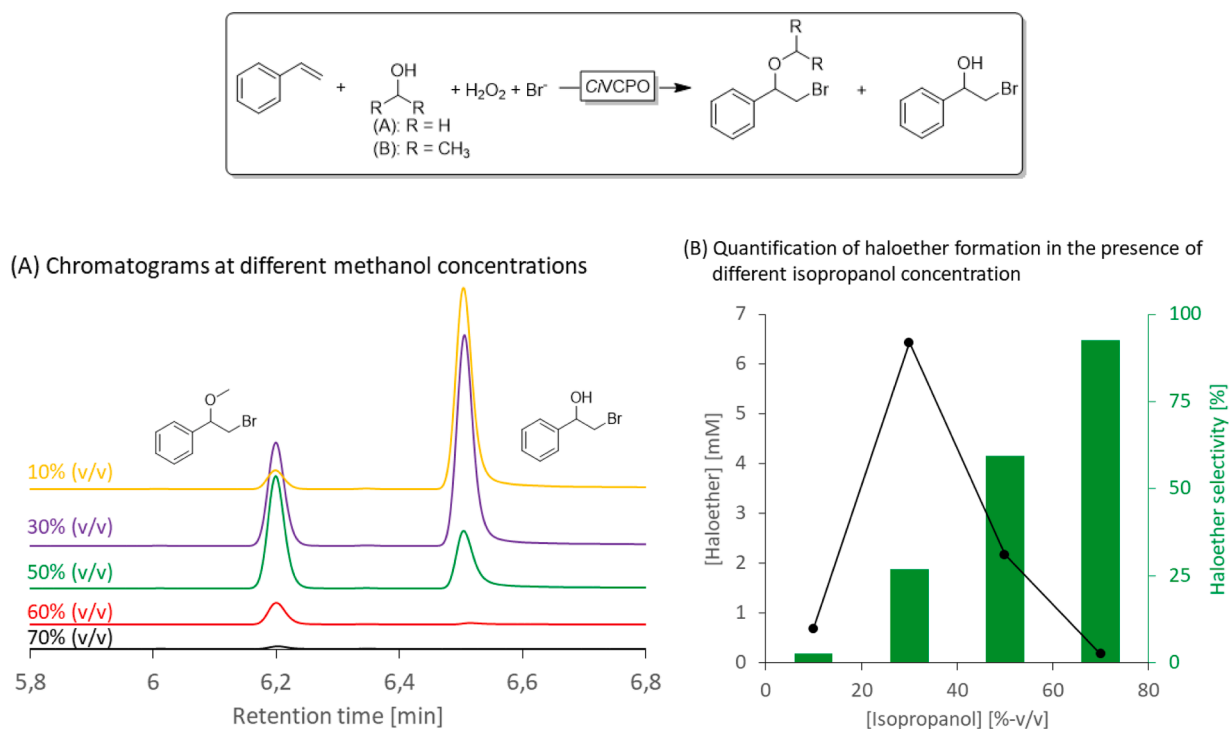
Fig. 1. Product distribution of the chemoenzymatic haloetherification using isopropanol as a nucleophile at different ratio's water and isopropanol. (A) The concentrations of the haloethers and halohydrin products determined by gas chromatography in the presence of different methanol concentrations. (B) Quantitative analysis of the haloetherification reaction in the presence of different isopropanol concentrations. Conditions: [styrene] = 30 mM, [H_2O_2] = 100 mM, [KBr] = 100 mM, [CiVCPO] = 500 nM, pH 6.0 (NaPi buffer, 100 mM), T = 30 °C, t= 5 h.

catalytic activity of the wild type CiVCPO in the presence of very high cosolvent concentrations.

The regioselectivity of the haloether formation was strictly Markovnikov-type as only the 2-halo-1-alkoxy product detectable with the analytical methods applied (i.e. GC, NMR, TLC, Figure S5).

Though suboptimal from a selectivity point-of-view, we used 30% (v/v) concentrations to further explore the synthetic potential of the proposed chemoenzymatic haloetherification reaction (Fig. 2). Primary, secondary, tertiary alcohols and phenol yielded the desired haloethers in
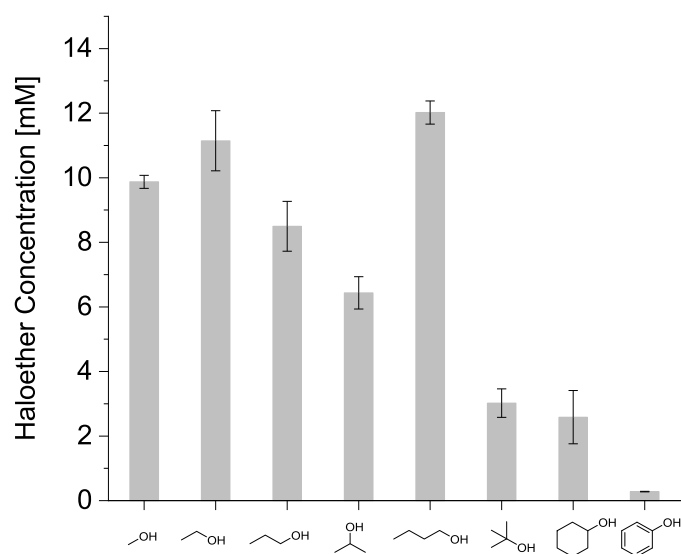
varying concentrations (Figures S6–10). Primary alcohols generally gave the highest haloethers yields which can most likely be attributed to steric reasons.

To explore the range of haloethers obtainable through the proposed chemoenzymatic procedure, we investigated a series of different alkenes and alcohol nucleophiles (Fig. 3 and Figures S11–96). These reactions were performed on semi-preparative (3 mmol) scale. Isolation and purification of the products was straightforward by simple extraction from the aqueous reaction media using ethyl acetate, followed by a single chromatographic step using 1% ethyl acetate in petroleum ether as eluent. Styrene derivates were generally transformed into the desired haloethers in acceptable isolated yields. No haloether-regioisomers other than the expected 2-halo-1-alkoxy regioisomers were observed. Generally, electron-rich starting alkenes were preferred whereas the previously observed reactivity order 1°>2°>3° alcohols was confirmed. Strongly electron-withdrawing substituents apparently deactivated the C=C-bond and no product could be isolated using CF_3^- or NO_2^--substituted styrenes (Figure S97). Aliphatic, cyclic and alicyclic alkenes were also generally converted.

Next to bromide, also chloride or iodide could be used as hypohalite source (Fig. 4 for some selected examples). Quite expectedly, the product formation rate with iodide was by far the highest and with chloride somewhat slower than with bromide (Figure S98).

As mentioned above, haloethers represent excellent starting materials for further derivatisation. Nevertheless, for some applications, removal of the halogen may be desirable. We therefore evaluated a variation of a previously reported catalytic deselenation reaction. Indeed, reductive dehalogenation of some selected chlorides (**31–32**), bromides (**33–34**) and iodides (**35–36**) proved to be straightforward using n-Bu$_3$SnH/AIBN with yields between 10.7% and 95.3% (Fig. 5 and Figures S99–104).

Fig. 2. Alcohol scope of the chemoenzymatic haloetherification reaction. [Styrene] = 30 mM, [alcohol] = 30% or [phenol] = 500 mM, [H_2O_2] = 50 mM, [KBr] = 50 mM, [CiVCPO] = 500 nM, T = 30 °C, t= 5 h, pH 5.0 (Citrate buffer, 100 mM).
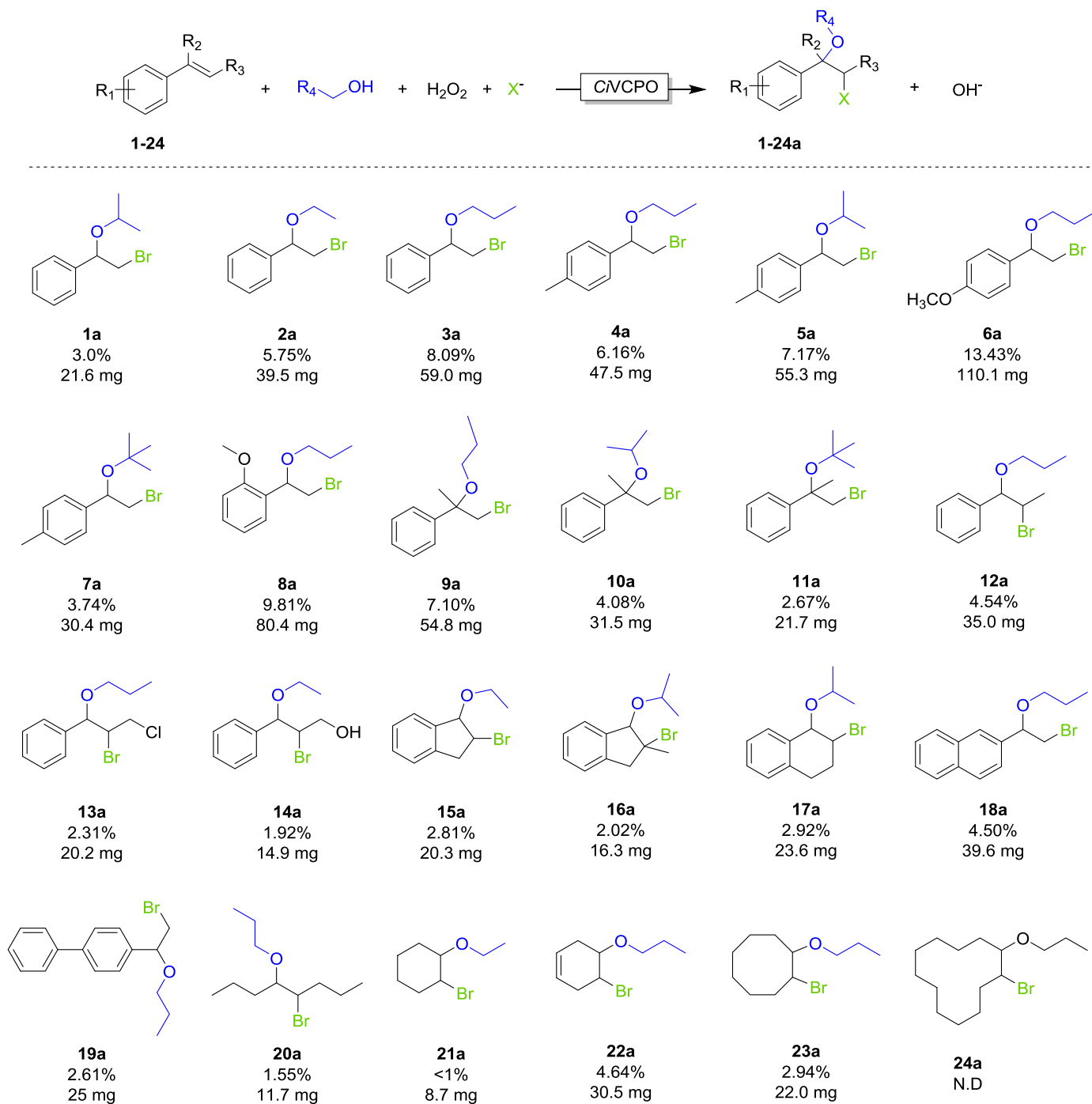
Fig. 3. Semi-preparative haloetherification of selected alkenes. General conditions: [alkene] = 30 mM, [alcohol] = 30% (v/v), [H_2O_2] = 50 mM, [KBr] =100 mM, [*Ci*VCPO] = 500 nM (0.8 × 10^{-3} mol %), pH 6.0 (NaPi buffer, 100 mM), 30% of alcohol, *T* = 30 °C, *t*= 6 h, 50 mL reaction scale.

Conclusions

Overall, with the present contribution we propose a chemoenzymatic approach to synthesize haloethers from alkenes and alcohols. Particularly, the mild reaction conditions and the very high catalytic performance of the biocatalysis are attractive. Today, despite the widespreadness of ethers in a wide variety of natural products,[20] enzymatic ether synthesis still represents a 'white spot' in the reaction portfolio with few preparative examples known so far such as the squalen-hoppene cyclases.[21][21]

Admittedly, the efficiency of the current setup is still limited by the competing hydrolysis of the halonium ion resulting in halohydrins.

Nevertheless, we demonstrate that the selectivity towards ether formation is affected by the ratio of water to alcohol nucleophile. Therefore, further investigations will focus both on the development of synthetic procedures using water-limited reaction media (e.g. natural deep eutectic solvents) and identifying more solvent-robust variants of *Ci*-CPO to perform the reactions in the presence of even higher alcohol concentrations.

Author contributions

S.C., J.Z. and Z.Z performed the experiments and analysed data. Z.D., Q.W. and R.W. analysed experimental data. F.H. and W.Z. promoted the
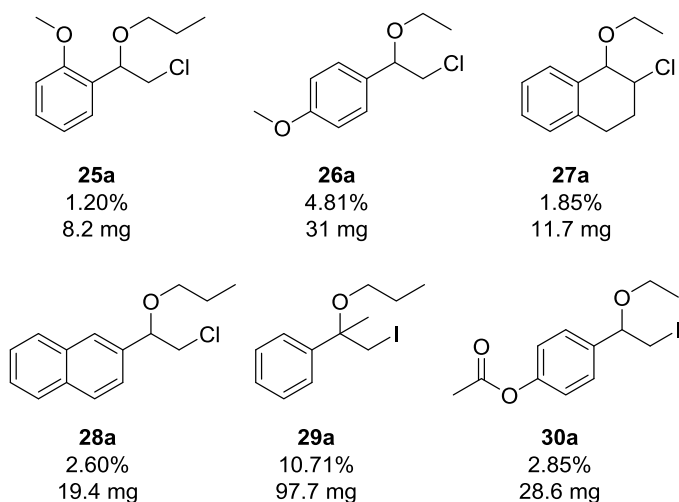
25a
1.20%
8.2 mg

26a
4.81%
31 mg

27a
1.85%
11.7 mg

28a
2.60%
19.4 mg

29a
10.71%
97.7 mg

30a
2.85%
28.6 mg

Fig. 4. Isolated Cl- and I-ethers between styrene and alcohols. Conditions: [alkene] = 30 mM, [alcohol] = 30% (v/v), [H_2O_2] = 50 mM, [KBr] = 100 mM, [*Ci*VCPO] = 500 nM (0.8 × 10^{-3} mol %), pH 6.0 (NaPi buffer, 100 mM), 30% alcohol already specified 30 °C, 6 h, 50 mL reaction scale.

31
10.7%

32
81.0%

33
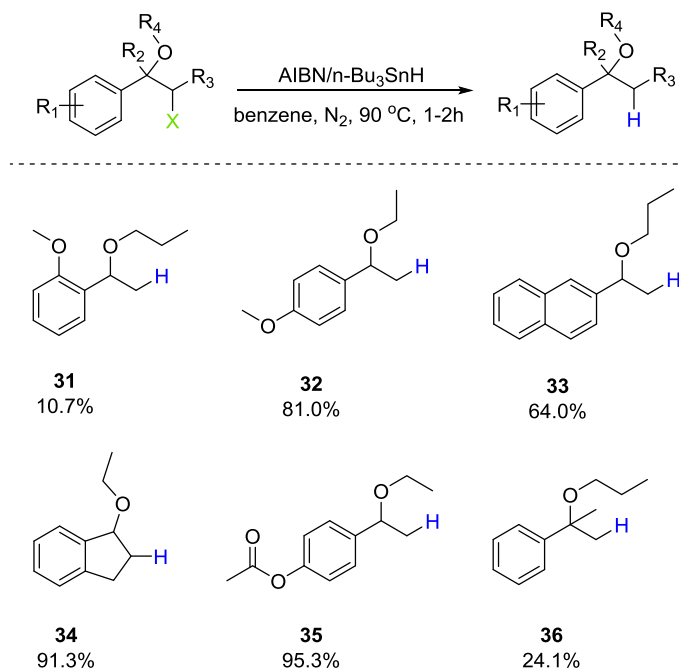64.0%

34
91.3%

35
95.3%

36
24.1%

Fig. 5. Reductive dehalogenation of some haloetherss synthesised in the chemoenzymatic haloethersification reaction: dechlorination (31 & 32), debromination (33 & 34) and deiodination (35 & 36). Reaction conditions: 0.083 mmol of the substrate, 0.25 mmol of n-Bu$_3$SnH and 0.25 mmol of AIBN were reacted for 1–2 h at 90 °C under N$_2$ atmosphere, 3 mL reaction scale.

concept and co-wrote the manuscript. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competition of interest.

CAS

S.C., J.Z. and Z.Z. performed the experiments and analysed data. Z.

D., Q.W. and R.W. analysed experimental data. F.H. and W.Z. promoted the concept and co-wrote the manuscript. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mcat.2021.112061.

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